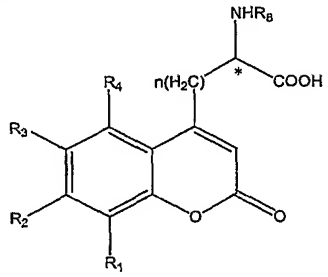
CLAIMS

1. A method for manufacturing an optically pure coumaryl L- or D- amino acid having the following formula (I) :



(I),

wherein :

(i) n is an integer ranging from 1 to 2 ;

(ii) R_1 represents H, halogen, alkyl, acyl, nitrile, sulfonate, aminosulfonyl, carbonyl and carbamoyl, OH, O- or N- substituted by alkyl or acyl group ;

(iii) R_2 represents H, halogen, alkyl, acyl, nitrile, sulfonate, aminosulfonyl, carbonyl and carbamoyl, OH, O- or N- substituted by alkyl or acyl group ;

(iv) R_3 represents H, halogen, alkyl, acyl, nitrile, sulfonate, aminosulfonyl, carbonyl and carbamoyl, OH, O- or N- substituted by alkyl or acyl group ;

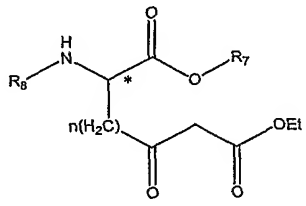
(v) R_4 represents H, halogen, alkyl, acyl, nitrile, sulfonate, aminosulfonyl, carbonyl and carbamoyl, OH, O- or N- substituted by alkyl or acyl group ;

(vi) R_8 represents a hydrogen atom or a protective group; and

(vii) "*" represents the position of an asymmetric carbon atom;

wherein said method comprises the step of :

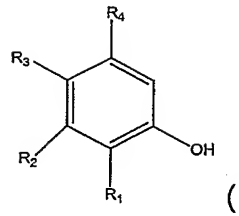
(b) reacting the L-amino acid β-ketoester of the following formula (1) :



(1)

wherein R_7 and R_8 mean, independently one from each other, a hydrogen atom or a protective group,

with a substituted phenol of the following formula (11) :



(11)

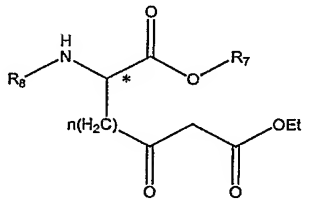
in the presence of methanesulfonic acid, for obtaining the compound of formula (I).

2. The method of claim 1 wherein, in the compound of Formula (I), R_8 means a hydrogen atom and said compound consists of an ammonium salt with an anionic compound.

3. The method of claim 2, wherein the anionic compound is selected from the group consisting of Cl^-, Br^-, I^-, CH_3SO_3, $CF_3CO_2^-$, $CF_3SO_3^-$.

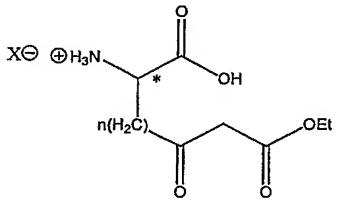
4. The method of claim 1 wherein, for the compound of formula (1), n is an integer ranging from 1 to 2.

5. The method of claim 1 wherein, for the compound of formula (1), n means 1 and step (b) comprises the following steps :
(b1) hydrogenolysis of the compound of formula (1)



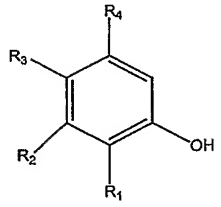
(1)

in the presence of catalytic palladium, for obtaining the compound of formula (2)



(2); and

(b2) reacting the compound of formula (2) with the compound of formula (11)



(11)

in the presence of methanesulfonic acid, for obtaining the compound of formula (I).

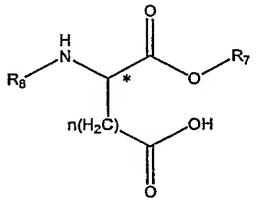
6. The method of claim 1 wherein, for the compound of formula (1), R_7 means a hydrogen atom and R_8 means a protective group.

7. The method of claim 1 wherein, for the compound of formula (1), R_7 and R_8 both mean a hydrogen atom.

8. The method of claim 1 wherein, for the compound of formula (1), R_7 and R_8 both mean, one independently from the other, .a protective group.

9. The method of any one of claims 1 to 6, wherein the L-amino acid β-ketoester compound of formula (1) is obtained through the steps of :

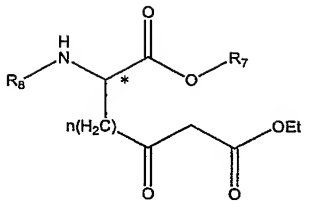
(a1) subjecting a protected amino acid of the following formula (IV)



(IV)

by treatment with carbonyldiimidazole; and

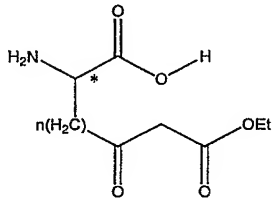
(a2) reacting the activated compound obtained at step (a) with a salt of monoethyl malonic acid, for obtaining the amino acid β-ketoester compound of formula (1)



(1)

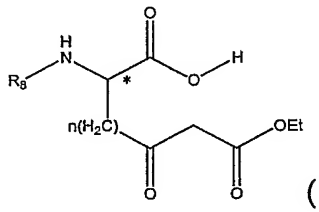
10. The method of claim 4, wherein the amino acid β-ketoester of formula (1), is obtained through the steps of :

(aa1) subjecting a protected amino acids β-ketoester of the following formula (1A) :



(1A)

to a reaction with a protective group, whereby obtaining the following amino acid β-ketoester of formula (1):

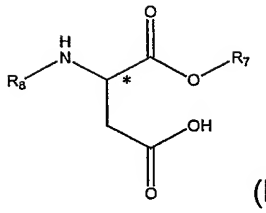


(1)

wherein R_8 means a protective group.

11. The method of claim 4, wherein the amino acid β-ketoester of formula (1) is obtained through the steps of :

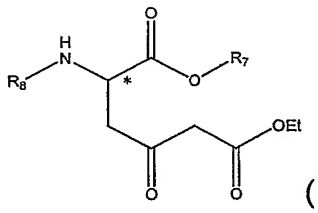
(ab1) reacting a protected aspartic acid residue of the following formula (R_8-Asp-R_7)



(R_8-Asp-R_7)

[by treatment with carbonyldiimidazole]; and

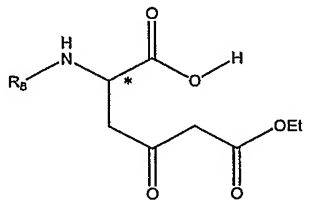
(ab2) reacting the activated compound obtained at step (ab1) with a salt of monoethyl malonic acid, for obtaining the amino acid β-ketoester compounds of formula (1):



(1)

wherein R_7 and R_8 both mean, one independently from the other, a protective group; and

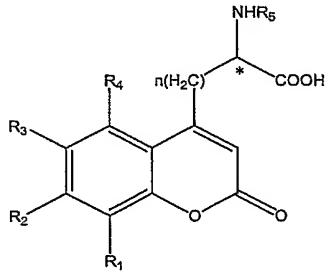
(ab3) removing the R_7 protective group, whereby obtaining the aminoacid β-ketoester compound of formula (1):



12. A method for manufacturing an optically pure coumaryl L- or D-amino acid that is protected on its amino group, wherein said method comprises the steps of :

(A) obtaining the compound of formula (I) according to the method of claim 1, wherein R_7 and R_8 mean, one independently from the other, a hydrogen atom;

(c) reacting the compound of formula (I) obtained at step (A) with the appropriate protective group, whereby obtaining the protected compound of formula (II) :



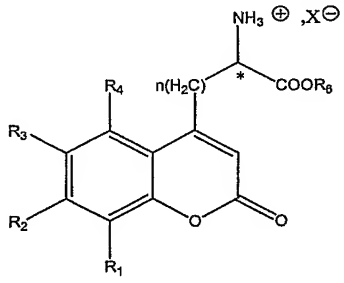
(II)

wherein R_5 is a protective group.

13. A method for manufacturing an optically pure L- or D- coumaryl amino acid that is protected on its carboxyl group, wherein said method comprises the steps of :

(A) obtaining the compound of formula (I) according to the method of claim 1; and

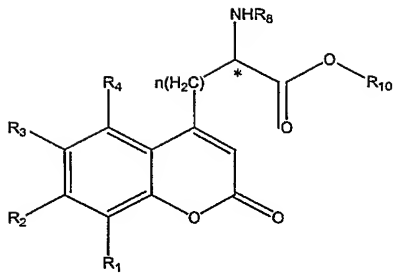
(d) reacting the compound of formula (I) obtained at step (A) with the appropriate protective group, whereby obtaining the protected compound of formula (III) :



(III)

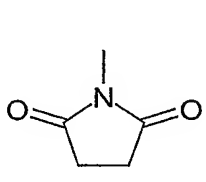
wherein R_6 is a protective group.

14. A method for manufacturing an optically pure activated L- or D-coumaryl amino acid of formula (X) :

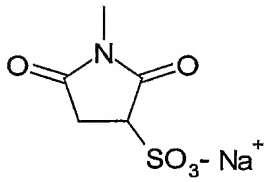


(X),

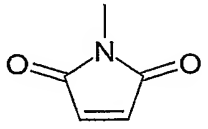
wherein R_1, R_2, R_3 and R_4 have the same meanings as in claim 1, R_8 is a protective group and R_{10} is an activator group selected from the group consisting of :



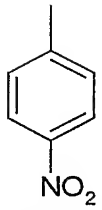
succinimidyl ester



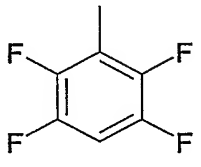
sulfosuccinimidyl ester, sodium salt



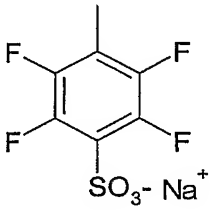
maleimidyl ester



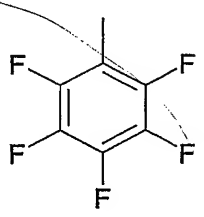
4-nitrophenyl ester



2,3,5,6-tetrafluoro-phenyl ester



4-sulfotetrafluorophenyl ester, sodium salt



pentafluorophenyl ester

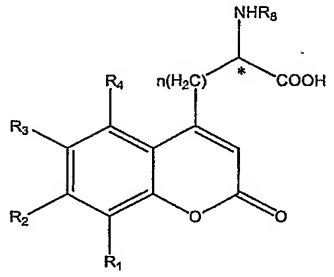
said method comprising the steps of :

(x1) obtaining the compound of formula (I) according to the method of claim 1, wherein R_8 means a protective group and R_7 means a hydrogen atom; and

(x2) reacting the compound of formula (I) obtained at step (x1) with an activator group [R8-OH], whereby the optically pure activated coumaryl amino acid of formula (X) is obtained.

15. The method according to claim 14, wherein group R_8 is selected from the group consisting of Fmoc, Boc and Cbz.

16. An optically pure L- or D- coumaryl amino acid salt the following formula (I) :



(I),

wherein :

(i) n is an integer ranging from 1 to 2 ;

(ii) R_1 represents H, halogen, alkyl, acyl, nitrile, sulfonate, aminosulfonyl, carbonyl and carbamoyl, OH, O- or N- substituted by alkyl or acyl group ;

(iii) R_2 represents H, halogen, alkyl, acyl, nitrile, sulfonate, aminosulfonyl, carbonyl and carbamoyl, OH, O- or N- substituted by alkyl or acyl group ;

(iv) R_3 represents H, halogen, alkyl, acyl, nitrile, sulfonate, aminosulfonyl, carbonyl and carbamoyl, OH, O- or N- substituted by alkyl or acyl group ;

(v) R_4 represents H, halogen, alkyl, acyl, nitrile, sulfonate, aminosulfonyl, carbonyl and carbamoyl, OH, O- or N- substituted by alkyl or acyl group ;

(vi) R_8 represents a hydrogen atom or a protective group; and

(vii) "*" represents the position of an asymmetric carbon atom;

provided that said coumaryl aminoacid does not consist of a compound wherein:

- R_1, R_3 and R_4 mean simultaneously a hydrogen atom, and R_2 means a methoxy group, or

- R_1 and R_4 mean simultaneously a hydrogen atom, and R_2 and R_3 both mean a methoxy group.

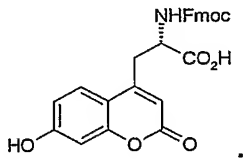
17. The optically pure L- or D- coumaryl aminoacid of claim 16, wherein R_8 means a hydrogen atom and said compound consists of an ammonium salt with an anionic compound.

18. The optically pure L- or D- coumaryl aminoacid salt of claim 16 wherein the anionic compound is selected from the group consisting of Cl^-, Br^-, I^-, $CH_3SO_3^-$, $CF_3CO_2^-$, $CF_3SO_3^-$.

19. The optically active coumaryl amino acid according to claim 16, which is selected from the group consisting of :

- (1S)-1-carboxy-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)-ethyl ammonium trifluoroacetate,
- (1R)-1-carboxy-2-(7-methoxy-2-oxo-2H-chromen-4-yl)-ethyl ammonium trifluoroacetate,
- (1S)-1-carboxy-2-(6-chloro, 7-hydroxy-2-oxo-2H-chromen-4-yl)-ethyl ammonium trifluoroacetate,
- (1S)-1-carboxy-2-(7-ethoxy-2-oxo-2H-chromen-4-yl)-ethyl ammonium trifluoroacetate,
- (1S)-1-carboxy-2-(5-hydroxy, 7-methoxy-2-oxo-2H-chromen-4-yl)-ethyl ammonium trifluoroacetate; and
- (1S)-1-carboxy-2-(7-hydroxy, 5-methoxy-2-oxo-2H-chromen-4-yl)-ethyl ammonium trifluoroacetate,

20. A compound of formula (I) according to claim 1 consisting of the compound of the following formula :



21. A compound of formula (II) according to claim 12 which is selected from the group consisting of :

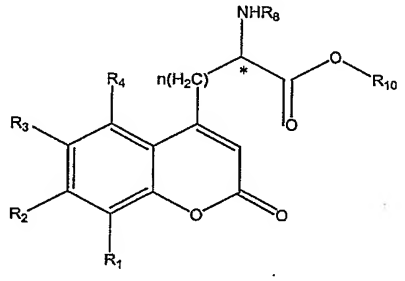
- (2S)-2-Fmoc-amino-3-(7-methoxy-2-oxo-2H-chromen-4-yl)propionic acid,
- (2S)-2-Cbz-amino-3-(7-methoxy-2-oxo-2H-chromen-4-yl)propionic acid,

- (2S)-2-Boc-amino-3-(7-methoxy-2-oxo-2H-chromen-4-yl)propionic acid; and

- (2R)-2-Fmoc-amino-3-(7-methoxy-2-oxo-2H-chromen-4-yl)propionic acid.

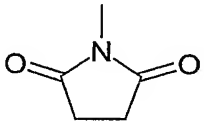
22. A compound of formula (III) according to claim 13 which consists of (1S)-1-benzyloxycarbonyl-3-(7-methoxy-2-oxo-2H-chromen-4-yl)-propyl ammonium trifluoro-acetate.

23. A compound of formula (X) :

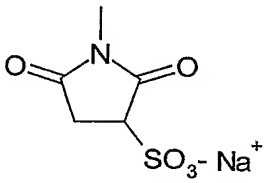


(X),

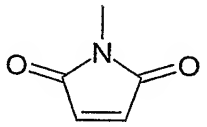
wherein R_1, R_2, R_3 and R_4 have the same meanings as in claim 1, R_8 is a protective group and R_{10} is an activator group selected from the group consisting of :



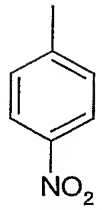
succinimidyl ester



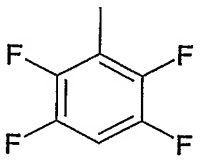
sulfosuccinimidyl ester, sodium salt



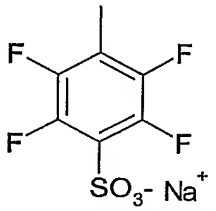
maleimidyl ester



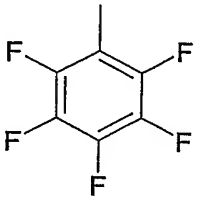
4-nitrophenyl ester



2,3,5,6-tetrafluoro-phenyl ester



4-sulfotetrafluorophenyl ester, sodium salt



pentafluorophenyl ester

24. The compound of formula (X) according to claim 23, wherein group R8 is selected from the group consisting of Fmoc, Boc and Cbz.

25. A kit for manufacturing a fluorescent polypeptide, wherein said kit comprises one coumaryl amino acid derivative selected from the compounds of formula (I), (II), (III) and (X), as defined in claims 1, 12, 13 and 23.

26. A method for the synthesis of an optically active polypeptide wherein said method comprises at least one step of incorporating an optically active coumaryl amino acid selected from the compounds of formula (I), (II), (III) and (X) within the amino acid chain.

27. An optically active polypeptide which contains in its amino acid chain an optically active coumaryl amino acid of formula (I).

28. An *in vitro* assay kit comprising an optically active polypeptide, which polypeptide contains in its amino acid chain an optically active coumaryl amino acid of formula (I).